eDNA Barcoding: Using Next-Generation Sequencing of Environmental DNA for Detection and Identification of Cetacean Species

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LONG-TERM GOALS

We are developing next-generation sequencing and digital (d)PCR methodology for detection and species identification of cetaceans using environmental (e)DNA collected from seawater.

Referred to here as '(e)DNA barcoding', this new methodology can be used to [a] detect and identify the presence of (non-vocal) cetaceans in the field and [b] identify the species of unknown cetacean vocalizations to improve the accuracy of ongoing U.S. Navy passive acoustic monitoring efforts.

The development of this methodology involves optimizing and modifying a number of conventional and 'next-generation' laboratory protocols, as well as initial field-testing under a relatively controlled environment. In the first year, we have conducted a series of (e)DNA sampling experiments in the vicinity of killer whales *Orcinus orca* near San Juan Island in Puget Sound during two, weeklong periods of fieldwork in August and September, 2015. The regular habits of the southern residents in these semi-enclosed waters and their well-characterized vocalizations allowed us to track pods and collect seawater at various distances and time periods after the path of the whales. The known differences between mitochondrial (mt)DNA lineages of killer whales will be used to demonstrate the potential for identification of ecotypes, as well as species. The fieldwork was completed successfully and laboratory analyses are now underway to quantify detection of (e)DNA during the serial-sampling experiments and confirm species identification using metabarcoding by next-generation sequencing.

In the second year, we will move to an open-ocean environment, supported by a fixed or towed acoustic detection array to locate cetaceans. The location of these open-ocean tests will be decided dependent on the (e)DNA thresholds estimated in the first year and in consultation with the Navy.

OBJECTIVES

1) Develop methods for (e)DNA sampling and extraction and design PCR primers for (e)DNA barcoding of cetaceans.
2) Conduct field work to collect (e)DNA from the vicintiy of killer whales around San Juan Islands, at known distances and times after the passage of the whales.

3) Analyze (e)DNA samples collected from vicinity of killer whales by extraction, amplification, quantification (by digital PCR) and sequencing by conventional and enext generation metabarcoding.

4) Conduct fieldwork to collect (e)DNA in an open-ocean environment to be decided dependent on the (e)DNA thresholds estimated in the first year and in consultation with the Navy.

5) Analyze (e)DNA samples collected during open-ocean sampling by amplification, quantification (by digital PCR) and sequencing by conventional and next generation metabarcoding

6) Finalize report on detection and indentification of cetacean species from (e)DNA and dissemate results by presentation at

APPROACH

(e)DNA will be collected by filtration from seawater sampled in the vicinity of cetaceans (Fig 1). Detection and quantification of (e)DNA will be conducted by digital (d)PCR and species identification will be conducted by next-generation 'metabarcoding'. Next-generation sequencing of the pooled amplicons will provide 100,000s of short reads (up to 250 base pairs in length) from each (e)DNA sampling experiment, improving the reliability and sensitivity of species identification, even when multiple species are present. The unique amplicon sequences (haplotypes) can be matched back to the reference database or searched against GenBank for species identification. By using individually indexed sequencing adaptors, we can multiplex many experiments in a single next-generation sequencing run, greatly reducing the cost of species monitoring.

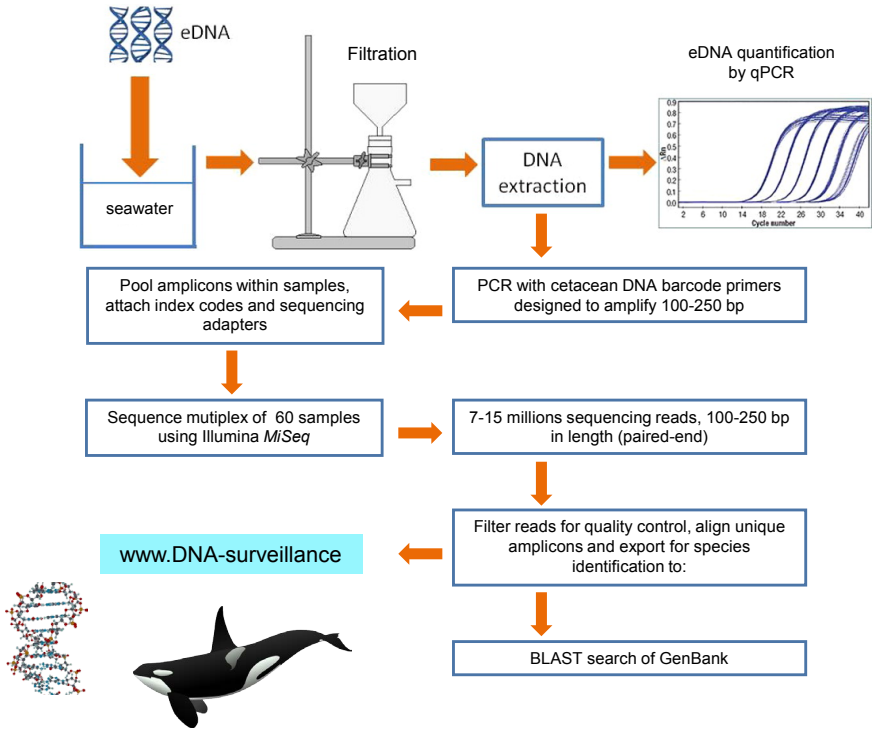

Figure 1. The workflow for (e)DNA barcoding of cetacean by next-generation sequencing, as proposed. We have subsequently obtained access to a digital (d)PCR instrument (in addition to qPCR) to to enhance detection and quantification of target DNA.

WORK COMPLETED

We have conducted a series of (e)DNA sampling experiments in the vicinity of killer whales *Orcinus orca* near San Juan Island in Puget Sound during two weeklong periods of field work in August and September, 2015. During each trip, we were hosted at the Friday Harbor Laboratories (University of Washington) and surveyed waters around the San Juan Islands from one of the laboratories small vessel (Fig 2). We collected seawater from the vicinity of killer whales on 26 encounters during the August field effort and on 16 encounters during the September fieldwork. We conducted both single-sample and serial sampling, starting 200 m behind the pod (to comply with local whale watching regulations). For serial sampling, we used a GPS drift buoy to maintain our position in the water mass after the whales passed (Fig 3).

The initial surveys involved bottle samples of near-surface water (2 liters) followed by filtering with 0.2 or 0.4 micron polycarbonate filters. To help account for the spatial variability in (e)DNA, we have also experimented with vertical tows using a 35-micron plankton net and horizontal tows using a 65-micron plankton net. Laboratory analyses are now underway to extract the (e)DNA from the filers and optimize the amplification of (mt)DNA for species identification using a suite of PCR primers designed from a comprehensive reference database of sequences from cetacean species.

Figure 2: Deployment of a hydrophone and a drift buoy (red sphere on port side of boat) and passage of a whale-watching vessel, during (e)DNA sampling of killer whales in San Juan Islands, September 2015. Photograph courtesy of Jeanne Hyde.

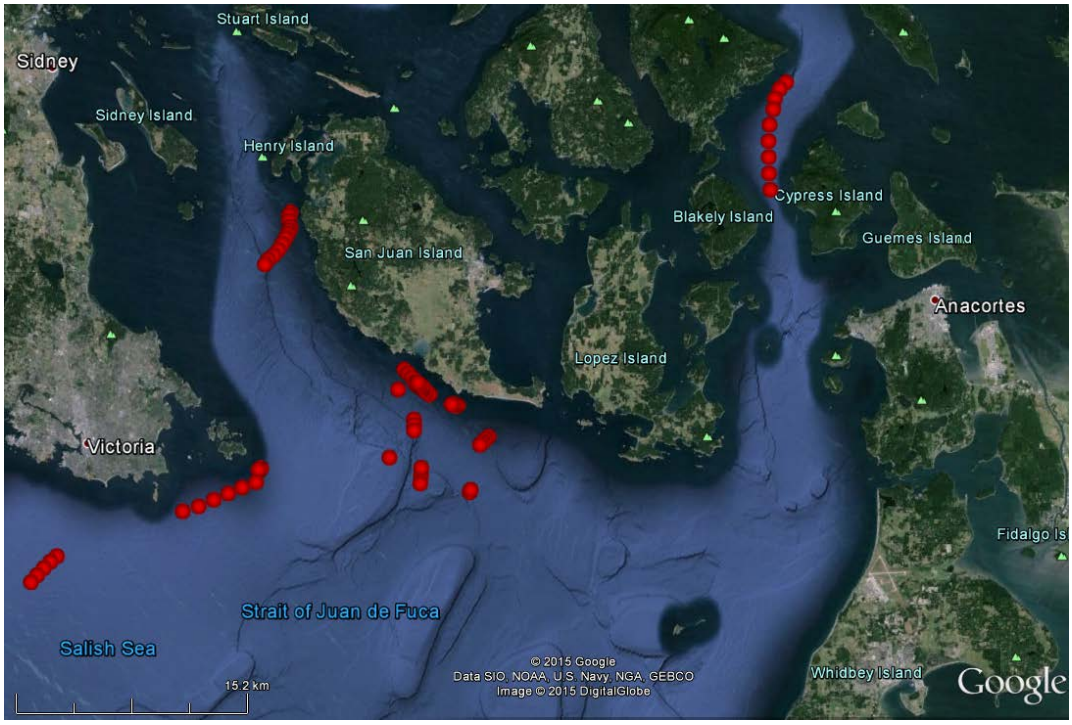

Figure 3: The location of (e)DNA serial sampling encounter from killer whales in the San Juan Islands during August and September 2015. The red dots show the position of the drift buoy in 10-minute intervals, after placing ourselves in an initial position after the passage of the whales.

RESULTS

The first phase of this project has focused on collection of seawater after the passage of differing number of killer whales in the semi-enclosed water of the Salish Sea, around the San Juan Islands. The logistics of this 'natural experiment' were very success, allowing us to collect serial samples, at a range of distances and times, after the passage of killer whales from a range of group sizes.

IMPACT/APPLICATIONS

If successful under open-ocean conditions, routine (e)DNA sampling could complement the interpretation of acoustic and visual surveys now routinely used to monitor cetacean habitat, especially for rare or cryptic species like beaked whales. In general, next-generation sequencing technology and informatics are advancing rapidly and sequencing costs are dropping rapidly, promising to make ubiquitous DNA sequencing for surveys of biodiversity more efficient and affordable in the near future.

RELATED PROJECTS

None to date.